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## The Role of Selenium in Glutathione Peroxidase

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# Summary

The role of selenium as an antioxidant, in particular as a key component in the enzymatic activity of glutathione peroxidase, was described and analyzed with a computational methodology, employing state-of-the art quantum mechanical techniques combined with classic calculations. Density functional theory methods were the main approach employed to obtain structural, energetic and mechanistic information on model systems. To include the effect of part of the systems that were left out of the QM calculations, classic molecular dynamics simulations were carried out using a recently developed force field tailored to effectively model protein structures. Finally, the application of quantitative models for energy decomposition (activation strain model and energy decomposition analysis) allowed an in-depth analysis of the formation of the reaction barriers and their underlying causes. These *in silico* techniques made the study of the intrinsic properties of selenium and of the other chalcogens possible.

Three scenarios were selected and tested: the ability of chalcogenides to form weak non-covalent bonds (Chapter 3), their thermodynamics and reactivity in  $S_N2$  substitution processes (Chapter 5) and their reactivity toward  $H_2O_2$  in redox reactions (Chapters 4 and 6).

Non covalent chalcogen bonds have stirred a lot of interest lately for their importance in protein interaction and their possible application to catalysis, ion

transport and rational drug design. A series of halogen substituted chalcogenides were chosen as model systems. Three small unsaturated organic molecules were selected as Lewis bases for the formation of the weak interaction. The resulting chalcogen- $\pi$  bond increases in strength as the chalcogen-halogen electronegativity difference becomes larger. This is due to a combination of electrostatic and orbital effects. The bond acceptor can also modify the strength of the interaction based on the energy of its HOMO: substrates with a high energy HOMO interact more favorably with the chalcogenides resulting in stronger bonds. These considerations can be applied to a broad range of chalcogen- $\pi$  complexes and will help understand their behavior and direct any rational design attempts towards the most favorable scenario.

Maximizing the efficiency of each step in a catalytic cycle is the keystone of the rational design of any catalyst and while the noncovalent interactions are predominant in the initial formation reactant complexes, the redox reactions in which selenium is involved, when acting as an antioxidant, require the breaking and formation of covalent bonds. The catalytic cycle of human glutathione peroxidase 4 (GPx4) was chosen as a model to determine which factors contribute to the enzymatic activity in order to establish the required features in an efficient artificial GPx mimic and to unravel the fundamental traits of the three chalcogens (S, Se and Te) which could be exploited to rationally design the most befitting molecule for a targeted application. Two steps of the whole GPx4 mechanism were thoroughly analyzed: the first oxidative reaction and the last reductive phase.

The oxidative part was studied with a combined classical/QM method in which the MD simulations served as a starting point for the subsequent DFT calculations. Results on an enzymatic cluster representing the active site show that selenium is thermodynamically favored over sulfur due to the weaker Se-H bond present in the initial structure that can more easily undergo the proton

transfer needed to begin the reaction. Moreover, tellurium displays a different mechanism and can act differently from the other chalcogens as the catalytic cycle of the semi-natural tellurium-GPx4 enzyme could pass through highly oxidized tellurium states.

The final reductive step was modeled as a nucleophilic substitution of a methyl chalcogenolate on a dimethyl dichalcogenide. The choice of such a small system allows the focus to be centered on the intrinsic properties of the different chalcogens employed (S, Se and Te) to evaluate their particular behavior. Results in the gas phase show how nucleophilic substitutions have always an addition-elimination mechanism with the formation of a three-center transition complex, but those at tellurium show a single-well profile, whereas those at the other chalcogens have a triple-well profile. The inclusion of the solvent in the calculations as a dielectric continuum, aims to simulate an environment closer to that of the *in vivo* enzyme. In the condensed phase mechanistic features of this reactions depend upon the chalcogen that undergoes nucleophilic attack: in the case of sulfur the reaction is seen to be an  $S_N2$ , in the case of tellurium it retains the single-well profile of the gas-phase, albeit with a less stabilized transition complex, and in the case of selenium a transitional mechanism is proposed which shows an energy profile with an almost flat central region inside which multiple intermediates and transition states are located. This mechanistic difference could be one of the key reasons why selenium is present in the active site of GPx, as the formation of the three-center complex seems to be unlikely in the enzyme due to the steric hindrance caused by the surrounding residues. Otherwise, the regeneration of the enzyme in the last reductive step would be greatly hampered since the nucleophilic attack at selenium, which would result in an unwanted thiol scrambling reaction, is thermodynamically favored over that at sulfur.

The modeling of enzymatic cycles can offer great insights on the “secrets” of the efficiency and effectiveness of these catalysts. However, transferring of

the information obtained for an enzyme to a smaller and easily synthesizable molecules that should reproduce the catalytic activity is never straightforward. A complete understanding of the processes in which these small mimics are involved is key to correctly translate and apply the results obtained for larger systems. Therefore in the final part of this Thesis an in-depth study of the reactivity of a very promising class of GPx mimics towards  $\text{H}_2\text{O}_2$  is detailed. Organic diselenides are extensively employed in synthesis as catalysts and show promising features to be employed as efficient antioxidant compounds in pharmacology and medicine. Recently, organic tellurides were also studied for their antioxidant properties, although the lack of a complete picture of the toxicology of these compounds limits their employment as therapeutic agents. The oxidation reaction of selected diselenides and ditellurides by hydrogen peroxide was investigated with DFT techniques. For the simplest compounds activation strain analyses were performed along the reaction coordinate from the starting reactant complex to the transition state. Results show an increased reactivity towards  $\text{H}_2\text{O}_2$  going from sulfur to tellurium leading to the formation of a sulfoxide/selenoxide/telluroxide and a very similar reaction profile for dimethyl and diphenyl chalcogenides. Further investigations on *para* substituted diphenyl diselenides and ditellurides show that, although the ring substituent has a modest effect on the oxidation barrier, electron donating moieties favor the oxidation process, decreasing the activation energy. Finally, the isomerization of the chalcogenoxide obtained after oxidation was investigated in light of the fact that the resulting anhydride seems to be an important intermediate to reach highly oxidized states that play a key role in the catalysis of redox reactions, at least in the case of diselenides. Computed barriers are always larger than those for the initial oxidation, with ditellurides having smaller isomerization barriers compared to diselenides. Moreover, the resulting anhydrides lie at a higher energy than the starting oxide making the reaction thermodynamically unfavorable.